588. *Isosbestic Points.*

By M. D. Cohen and E. Fischer.

The conditions for the occurrence of isosbestic points are considered. It is shown that, for a closed system, it is necessary that: (i) the spectra of the limiting systems (*e.g.*, the systems before and after reaction) intersect; and (ii) the changes in the concentrations of the various components are linearly related. When a chemical reaction is involved, the second condition implies that a single parameter is sufficient to specify uniquely the composition of the system at any time. Provided the above two conditions are satisfied, isosbestic points may be found with systems of any number of components. The existence or absence of isosbestic points provides a basis for distinguishing between systems whose compositions are defined by single reaction parameters, and those requiring more than one such parameter. Examples of this distinction are provided by simultaneous and consecutive reactions, respectively.

It is sometimes found that in the spectra of a multicomponent system there are wavelengths such that the absorption remains constant for all compositions of the system, provided the overall concentration is fixed. Each such point in the spectrum is called an isosbestic point. Various examples will be found in the Figures of this paper.

An early, brief discussion of such points was published by Scheibe.[1] Isosbestic points have been noted very frequently and conclusions about the number and types of species concerned have often been drawn from the presence or absence of such points but there appears to be no generalized treatment of the conditions necessary for the occurrence of such points, nor are these conditions always understood. As a result the conclusions drawn from the spectra are sometimes erroneous, or at least incorrectly based.

We here consider the conditions required for the occurrence of isosbestic points and then give examples.

The General Case.—In considering the general case we introduce de Donder and van Rysselberghe's parameter ξ, which defines the extent of reaction.[2] Let us take, for example, the reaction:

$$aA + bB + \ldots = pP + rR + \ldots \qquad (1)$$

or

$$0 = pP + rR + \ldots - aA - bB - \ldots \qquad (2)$$

or

$$0 = \sum_i \nu_i M_i,$$

where the M_i's are the various chemical species involved in the chemical reaction and the ν_i's are the corresponding stoicheiometric coefficients when the process is written as in (2).

Let n_i^0 be the initial number of moles of the i'th reactant and n_i the corresponding number at time *t*. de Donder and van Rysselberghe define

$$\xi = (n_i - n_i^0)/\nu_i \qquad (3)$$

Provided the changes in the numbers of moles of the various reactants are linearly related, as in (1) and (2), this parameter is independent of which reactant i is considered.

Let us now consider the system indicated by (1) above. The initial concentrations are $n_A^0, n_B^0, \cdots, n_P^0, n_R^0, \cdots$. Then by (3),

$$\xi = (n_A - n_A^0)/\nu_A = (n_B - n_B^0)/\nu_B = \cdots$$
$$= (n_P - n_P^0)/\nu_P = (n_R - n_R^0)/\nu_R = \cdots.$$

Thus,

$$n_A = n_A^0 - \xi a; \; n_B = n_B^0 - \xi b; \; \cdots\cdots; \; n_P = n_P^0 + \xi p; \qquad (4)$$
$$n_R = n_R^0 + \xi r; \; \cdots\cdots.$$

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[1] Scheibe, *Z. angew. Chem.*, 1937, **50**, 212.

[2] de Donder and van Rysselberghe, " Affinity," Stanford Univ. Press, 1936; see also Prigogine and Defay, " Chemical Thermodynamics," translated by D. H. Everett, Longmans, Green & Co., London, 1952, p. 10.

We assume, without loss of generality, that the concentration of A is the critical one, *i.e.*, that reaction (1) stops when the concentration of A falls to zero. At the end of the reaction, therefore,

$$\xi_\infty = -(0 - n_A{}^0)/a = n_A{}^0/a.$$

We consider that at a particular wavelength λ^1 the total absorption (optical density), $D_0{}^1$, of the initial system is the same as $D_\infty{}^1$, the absorption of the final system. Now

$$D_0{}^1 = n_A{}^0\varepsilon_A{}^1 + n_B{}^0\varepsilon_B{}^1 + \cdots\cdots + n_P{}^0\varepsilon_P{}^1 + n_R{}^0\varepsilon_R{}^1 + \cdots\cdot$$

where the ε's are the molecular extinction coefficients and we assume unit light path. At time t the total absorption is

$$D_t{}^1 = n_A\varepsilon_A{}^1 + n_B\varepsilon_B{}^1 + \cdots\cdots + n_P\varepsilon_P{}^1 + n_B\varepsilon_R{}^1 + \cdots\cdot$$

which gives, with (4),

$$D_t{}^1 = D_0{}^1 + \xi(p\varepsilon_P{}^1 + r\varepsilon_R{}^1 + \cdots\cdots - a\varepsilon_A{}^1 - b\varepsilon_B{}^1 - \cdots); \qquad (5)$$

further,

$$D_\infty{}^1 = D_0{}^1 + \frac{n_A{}^0}{a}(p\varepsilon_P{}^1 + r\varepsilon_R{}^1 + \cdots\cdots - a\varepsilon_A{}^1 - b\varepsilon_B{}^1 - \cdots). \qquad (6)$$

Also, for the system considered, $D_\infty{}^1 = D_0{}^1$. Thus the second term on the right-hand side of equation (6) must vanish. Since, however, $n_A{}^0$ is non-vanishing, the quantity within the parentheses must equal zero. Thus, from equation (5), $D_t{}^1 = D_0{}^1$, whatever the value of ξ.

Thus, in summary, provided that the stoicheiometric changes are all linearly related to one another, if the initial and final spectra intersect then all intermediate spectra will pass through the points of intersection. We see, in particular, that isosbestic points are by no means confined to two-component systems, as is commonly stated.[3,4] Systems whose spectra show such points can, however, be considered as being " pseudo-two-component " ones, there being only one " degree of freedom," the extent of reaction; the additional constraint on the system is applied by the stoicheiometric relation (1).

We have taken a reacting system as our model. However, the arguments given apply equally to a system which is caused to pass through a series of equilibrium states by the change in some factor such as temperature or solvent-composition. The treatment is valid also for mixtures of non-reacting species, provided that the changes in composition of these mixtures are made to satisfy stoicheiometric conditions analogous to those of equation (1).

Some cautionary points should be made at this stage: our arguments apply to a " closed " system, *i.e.*, one where no absorbing material is added to or removed from the space filled by the measuring light-beam. Thus the overall concentration is maintained constant. In experiments in which solvents of variable composition or various temperatures are used, results should be corrected to constant volume. Experiments which measure changes in composition resulting from different initial concentrations are not directly comparable; however, allowance can be made for the variation in overall concentration by plotting the apparent molecular extinction coefficient instead of the optical density.

Additional precautions need to be taken in experiments in which temperature or solvent composition is varied. Such changes will, in general, affect, not only the relative concentractions of the absorbing species, but also the shapes of the absorption bands of the individual components. The well-known sharpening of absorption bands at low temperatures is an example of this. If the changes are sufficiently drastic, therefore, the various extinction coefficients $\varepsilon_i{}^1$ are not constant and the conditions for occurrence of

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[3] Kortüm, " Kolorimetrie, Photometrie und Spektrometrie," Springer, Berlin, 1955, p. 34.

[4] Brügel, " Einführung in die Ultrarotspektroskopie," Steinkopff, Darmstadt, 1957, p. 292.

isosbestic points no longer obtain. Such points would then be expected to be found only over a limited range of variation of temperature or solvent. Thus Kortüm [5] presents the spectra of iodine in cyclohexane with varying concentrations of added dioxan. There are two long-wavelength bands, which intersect and are attributed to iodine and an iodine–dioxan complex. The spectra for a series of low dioxan concentrations (those shown in ref. 5 are for concentrations less than 5 mole %) all pass through the same point, whereas for high dioxan concentrations this is no longer so.

With these limitations in mind we now consider a number of systems classified according to their overall stoicheiometries. We use the double arrow to indicate any system in which molecules can be interconverted, be it at equilibrium or not.

$A \rightleftharpoons B$.—Systems of this group are amongst the best known of those showing isosbestic points. Examples are tautomeric molecules and those undergoing geometric isomerism. In some cases thermal interconversion of the isomers is possible; where the rate of thermal interconversion is low the molecules frequently undergo photo-isomerisation. The composition of the mixture of isomers at photo-equilibrium is determined by their absorption spectra and the wavelength of the light employed. Brode, Pearson, and Wyman [6] find isosbestic points in the spectra of diacetylindigo subjected to irradiation by light of various wavelengths. The spectral changes are attributed to *cis–trans*-isomerisation about the central C:C bond. Similarly, isosbestic points are found in *cis–trans*-isomerisation of azo-compounds [7] and anils.[8] Further examples may be found in the report of a Chemical Society Symposium.[9] Fig. 4a below describes a pertinent case.

Livingston [10] describes flash studies on some porphyrins in fluid solutions. Flashing converts an appreciable proportion of the molecules into their ground triplet state, from which they rapidly decay to the ground singlet state. When the singlet and the triplet absorption curves cross, the system, during decay, has isosbestic points coincident with these crossing points.

We may include in this group reactions of the type $A \rightleftharpoons B + X$, where X is a species which does not absorb at the wavelengths at which the spectra of A and B cross. A large number of systems fall into this sub-group and we classify further:

(*a*) $B \cdots HOR \rightleftharpoons B + ROH$, *where* ROH *is frequently the solvent, or a component of the solvent, and the dots indicate a hydrogen bond.* Perkampus [11] studied the " solvatochromy " of 3-hydroxy-1,2,4-trimethylphenazine, *i.e.*, the change in its absorption spectrum as the composition of the carbon tetrachloride–methanol solvent varies, as also with variation of temperature at each solvent composition. The main absorption band at 18,000 cm.$^{-1}$ (*ca.* 555 mμ) increases in intensity as the methanol content of the solvent increases and is attributed to solute hydrogen-bonded to aggregates of methanol molecules. The spectra show a number of isosbestic points.

Nagakura and Gouterman [12] found isosbestic points for the system α- or β-naphthol with suitable hydrogen-bond acceptors in n-heptane. In order to allow evaluation of the equilibrium constants the acceptor was always in excess relative to the donor. As above, the limiting spectra were attributed to the hydrogen-bonded and non-hydrogen-bonded species.

Baba [13] found a number of isosbestic points in the spectra of 4-hydroxyazobenzene in iso-octane as various amounts of ether or pyridine were added. The limiting spectra obtained were assigned to free solute molecules and to complexes in which these molecules

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[5] Ref. 3, p. 35.
[6] Brode, Pearson, and Wyman, *J. Amer. Chem. Soc.*, 1954, **76**, 1034.
[7] Fischer and Frei, *J. Chem. Phys.*, 1957, **27**, 328.
[8] Fischer and Frei, *J. Chem. Phys.*, 1957, **27**, 808.
[9] " Recent Advances in the Chemistry of Colouring Matters," *Chem. Soc. Special Publ.* No. 4, 1956.
[10] Livingston, *J. Amer. Chem. Soc.*, 1955, **77**, 2179.
[11] Suhrmann and Perkampus, *Z. phys. Chem.* (*Leipzig*), 1954, **2**, 290.
[12] Nagakura and Gouterman, *J. Chem. Phys.*, 1957, **26**, 881.
[13] Baba, *Bull. Chem. Soc. Japan*, 1958, **31**, 169.

are hydrogen-bonded to ether or pyridine. In this system there are three absorption bands which are displaced in the complex in directions apparently governed by whether the transitions are $n \longrightarrow \pi^*$ or $\pi \longrightarrow \pi^*$.

(*b*) *Formation of complexes other than hydrogen-bonded ones.* Ebara[14] studied solutions containing iodine and benzylideneanilines (anils). Measurements were made on solutions of fixed initial iodine concentration with various concentrations of anil, the latter always being in excess. For all anils it was found that the iodine maximum at *ca.* 513 mμ drops in intensity with increasing anil concentration, while a new band appears at lower wavelengths ($\lambda_{max.}$ at *ca.* 430 mμ), which is attributed to an iodine–anil complex. The anils themselves do not absorb in the region where these two curves cross. The isosbestic point falls near 495 mμ for all anols, although there are big differences in the intensities of the band of the complex from anil to anil.

For complexes of biological materials the binding forces are frequently of several types, so that the classification we use here is not strictly applicable. We consider an example where the main binding force is probably not of the hydrogen-bond type. Theorell and Bonnichsen,[15] and Theorell and Chance,[16] have studied the complex between the enzyme alcohol-dehydrogenase and reduced phosphopyridine nucleotide. The absorption spectra of the free and the bound nucleotide differ. The apparent molecular extinction coefficient at 328 mμ is constant for different initial concentrations of the reactants, *i.e.*, there is an isosbestic point at this wavelength. The authors use the optical density at this wavelength as a measure of the total concentration (free plus bound) of the nucleotide, while the concentration of bound nucleotide is obtained from the difference between this optical density and that at an appropriate second wavelength (354 mμ).

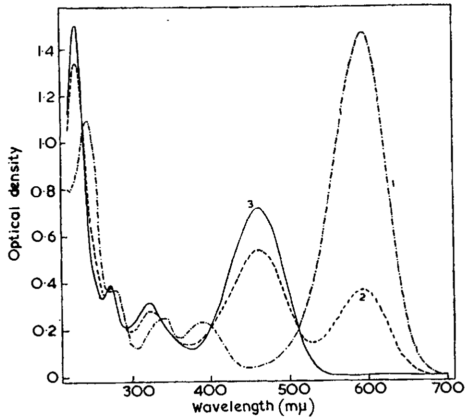

FIG. 1.

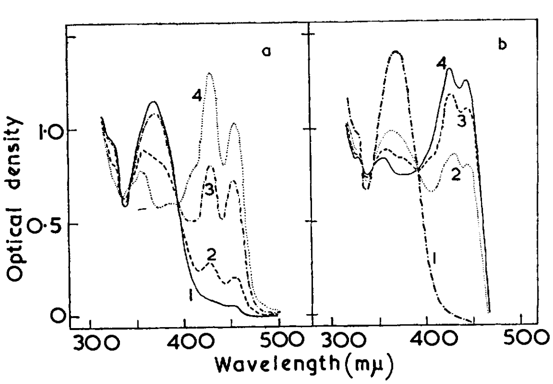

FIG. 2.

FIG. 1. Merocyanine (I) in ethanol–methanol (8·7 mg./l.; 10-mm. cell). (1) Original solution; (2) after addition of one equivalent of HCl; (3) after addition of two equivalents of HCl.

FIG. 2. (a) The spectrum of 2-hydroxy-1-naphthaldehyde anil in 2,2,5-trimethylhexane ($4{\cdot}37 \times 10^{-4}$ M; 2-mm. cell) at (1) 185°, (2) 173°, (3) 160°, and (4) 146° K. (b) The effect of solvent on the spectrum of 2-hydroxy-1-naphthaldehyde anil ($1{\cdot}67 \times 10^{-4}$ M; 10-mm. cell) in: (1) Trimethylhexane, (2) ethanol–trimethylhexane (1 : 2 v/v), (3) ethanol–trimethylhexane (2 : 1 v/v), and (4) ethanol.

(*c*) *Acids in equilibrium with their conjugate bases, i.e.*, $RH \rightleftharpoons R^- + H^+$. In general the acid and the base have different absorption characteristics so that such systems are

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[14] Ebara, *Bull. Chem. Soc. Japan*, 1960, **33**, 540.

[15] Theorell and Bonnichsen, *Acta Chem. Scand.*, 1951, **5**, 1105.

[16] Theorell and Chance, *Acta Chem. Scand.*, 1951, **5**, 1127.

pH-indicators. If the spectra of the acid and the base intersect there will be an isosbestic point for all compositions passed through as the pH varies.

Such behaviour is found, for example, in the spectra of quinone monoximes at various pH's.[17] The authors postulate that the tautomerization of the monoximes to nitrosophenols involves a preliminary ionization. Then, on the basis of their assignment of the two bands in the spectrum to the oxime and its conjugate ion, they correctly conclude that the amount of phenol form present must be negligible (see below on " consecutive reactions "). The authors point out, however, that this is not a proof of the assumed mechanism.

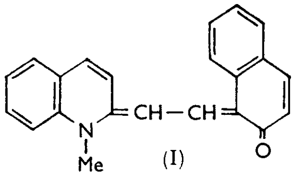
Fig. 1 shows spectra of the merocyanine (I) at various acid concentrations.[18] The bands at 595 and 455 mμ are due to the open-chain merocyanine and to its protonated species, respectively.

An interesting example given by Zanker [19] is that of the variation of the spectrum of Acridine Orange with pH. This system involves, in addition to the ionization equilibrium, a second equilibrium which is between the cation and its dimer: $R + H^+ = RH^+$; $2RH^+ = (RH)_2{}^{2+}$. The existence of the isosbestic point indicates that the dimerization equilibrium is established in times shorter than the time required for measurement, so that the reaction is represented by the equation: $R + H^+ = (1 - x)RH^+ + \frac{1}{2}x(RH)_2{}^{2+}$, where x describes the extent of dimerisation.

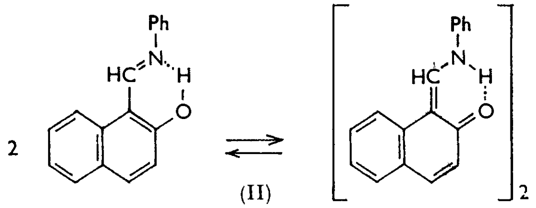
We point out also that for the two-component case there is no stoicheiometric distinction between a system whose composition varies as a result of the interconversion of two isomers, and a mixture whose composition is arbitrarily varied. It is common practice, for analytical purposes, to measure the spectra of mixtures of various compositions. This should give rise to isosbestic points when the spectra of the two components cross. Thus, Brügel [4] gives the spectra of various mixtures of butan-1-ol with butan-2-ol. There are two isosbestic points between 12 and 13 μ. This also illustrates the fact that isosbestic points are not confined to ultraviolet–visible (electronic) spectra.

$A \rightleftharpoons nB$.—To this category belong dissociating and polymerizing systems.

There are several ways of varying the extent of monomer–polymer interconversion. A common one is to change the initial concentration of monomer. In this case the system is not " closed " in the sense defined in the introductory section, and there will be no isosbestic points in plots of optical density against wavelength. However, there will be such points if the spectra are " corrected " by dividing the optical densities by the initial monomer concentration, *i.e.*, in plots of the apparent molecular extinction coefficient against wavelength.



(I)



(II)

Holst [20] found an isosbestic point in the spectra of Methylene Blue in aqueous solution, measured over 50-fold variation in concentration. The spectral changes were attributed to monomer–dimer interconversion. Similar results are obtained with aminoacridines,[21] which polymerise to an increasing extent with increasing concentration.

An alternative way of influencing the extent of conversion into polymer is to change

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[17] Havinga and Schors, *Rev. Trav. chim.*, 1950, **69**, 457.

[18] Heiligman, Hirshberg, and Fischer, unpublished results.

[19] Zanker, *Z. phys. Chem.* (*Frankfurt*), 1952, **199**, 225.

[20] Holst, *Z. phys. Chem.*, 1938, **182**, 321.

[21] Mataga, *Bull. Chem. Soc. Japan*, 1957, **30**, 375.

the temperature at constant concentration. The anils of 2-hydroxy-1-naphthaldehyde have $\lambda_{max.}$ for their long-wavelength absorption band in non-polar solvents at room temperature at about 370 mμ. Cooling these solutions lowers the intensity of this band whilst a new band appears in the visible region [22] (Fig. 2a). At constant temperature the degree of replacement of the 370 mμ band by the band in the visible region increases with increasing initial concentration. This change is attributed by the authors to polymerization. It is considered further that in the polymer the quinonoid tautomer of the anil is the stable entity (see II) and responsible for the absorption at low temperatures. A similar spectral change is observed on changing from a non-polar to a polar solvent at room temperature (Fig. 2b), but here the stabilization of the quinonoid tautomer is thought to be due to solvation by molecules of the polar solvent, and not to aggregation of solute molecules.

Another case of aggregation at low temperatures is given by Hausser.[23] *N*-Ethylphenazyl is paramagnetic in the crystal and in solution at room temperatures. At low temperatures the solution loses its paramagnetism and there are marked changes in the absorption spectrum, particularly in the near-infrared region (*ca.* 850 mμ). The author does not give a plot of the spectra for fixed initial concentrations at various temperatures. However, for various concentrations at constant low temperature the spectra show a number of clear isosbestic points. Hausser considers that the changes are due to formation of a diamagnetic dimer at low temperatures.

The classical examples of spectral changes associated with molecular aggregation are to be found in Scheibe's studies of photographic sensitizers. Most of these cases are, however, complex in that they involve a range of degrees of polymerization. There are, nevertheless, instances where isosbestic points are found for limited variation of the conditions, *i.e.*, concentration [24] or temperature.[25]

Simultaneous Reactions.—We consider the reaction (7) and assume that there are no complications such as autocatalysis. The ratio of the amount of C to that of B remains constant throughout the reaction ($= k_2/k_1$). In such a system, therefore, the concentrations of the species present are all linear functions of one another and there is a uniquely defined degree of advancement of the reaction. Thus, with the usual proviso of the crossing of the initial and the final absorption curve, one expects to find isosbestic points even when all three components absorb in the spectral range of interest.

$$\mathrm{B} \xleftarrow{k_1} \mathrm{A} \xrightarrow{k_2} \mathrm{C} \qquad (7)$$

We will show in the next section that this is not so for a system involving consecutive reactions. The presence or absence of isosbestic points should thus provide a simple way of distinguishing between simultaneous and consecutive reactions. In the absence of a suitable example for the " simultaneous " case we use an analogous case involving mixtures of three non-reacting compounds A, B, and C, all absorbing in the same spectral region. Equation (7) can be rewritten:

$$\mathrm{A} = \mathrm{B} + m\mathrm{C}, \qquad (8)$$

where m is a constant determining the ratio between B and C. We therefore took a solution of A and another containing B and C in a fixed ratio, and mixed them in various proportions, thus simulating various extents of reaction (8). Fig. 3 shows the results obtained with a series of such mixtures, together with the spectra of A, B, C, and (B + C).

Consecutive Reactions.—Despite the apparent generality of occurrence of isosbestic points there are certainly cases in which they will not occur. For example, for consecutive

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[22] Cohen, Hirshberg, and Schmidt in " Hydrogen Bonding," ed. Hadzi, Pergamon Press, London, 1959, p. 293.

[23] Hausser, *Z. Naturforsch.*, 1956, **11a**, 20.

[24] Scheibe, *Z. Elektrochem.*, 1948, **52**, 283.

[25] Scheibe, *Z. angew. Chem.*, 1939, **52**, 631.

reactions the points of intersection of successive curves lie at successively higher, or at successively lower, optical densities. Brode[26] calls the curve passing through the points of intersection of spectra 1 and 2, 2 and 3, 3 and 4, etc., an " isosbestic line."

Consider the reaction

$$A \longrightarrow B \longrightarrow C \qquad (9)$$

Let the spectrum of a solution of A of unit concentration intersect that of a similar solution of B at λ^1, *i.e.*, $\varepsilon_A{}^1 = \varepsilon_B{}^1$. If, at a given time, the concentration of B is x and that of C is y, then that of A is $(1 - x - y)$. The optical density at this time is:

$$\begin{aligned} D^1 &= (1 - x - y)\varepsilon_A{}^1 + x\varepsilon_B{}^1 + y\varepsilon_C{}^1 \\ &= (1 - y)\varepsilon_A{}^1 + y\varepsilon_C{}^1 \\ &= D_A{}^1 + y\,(\varepsilon_C{}^1 - \varepsilon_A{}^1). \end{aligned} \qquad (10)$$

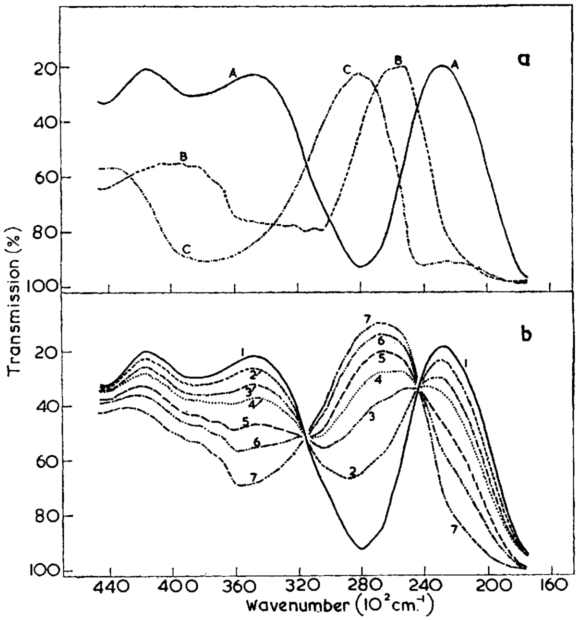

FIG. 3.

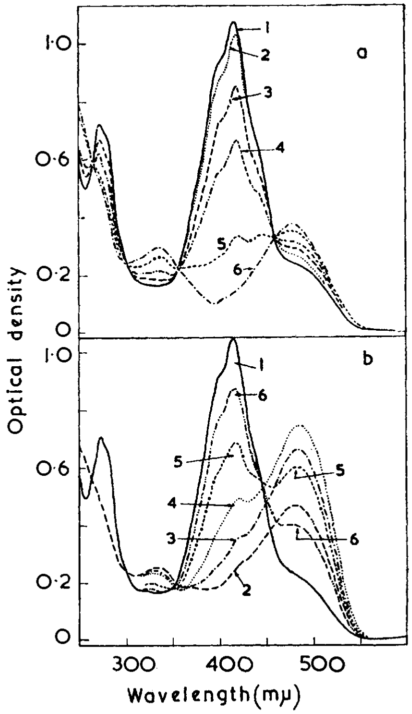

FIG. 4.

FIG. 3. (a) Absorption spectra, in 10-mm. cell, of compounds A (12 mg./l.), B (7 mg./l.), and C (4 mg./l.) in 2,2,5-trimethylhexane, (b) Curve 1, absorption spectra of a solution of A (12 mg./l.); curve 7, a solution of B and C (7 mg./l. and 4 mg./l., respectively), and mixtures of the two solutions in the following proportions: Curve 2, A/(B + C) = 5; curve 3, A/(B + C) = 2; curve 4, A/(B + C) = 1; curve 5, A/(B + C) = 0·5; curve 6, A/(B + C) = 0·2.

Designation of compounds: A, 1,4-naphthaquinone diphenylhydrazone; B, 4-dimethylaminoazobenzene; C, *p*-phenylenebis(azobenzene).

FIG. 4. Solution of compound (III) in ethanol–methanol (10 mg./l.; 10-mm. cell). (a) At −140°. (1) Before irradiation (*i.e.*, all *trans*); (2), (3), (4), (5) after photo-equilibration with light at 546, 365, 436, 405 mμ, respectively; (6) *cis*-isomer (computed). (b) (1) As in a (1); (2) as in a (5), heated to −120°; (3)—(6) spontaneous changes in the solution on storage at −120° (3,4) and then at −110° (5,6).

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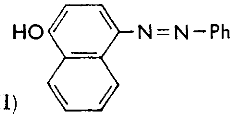
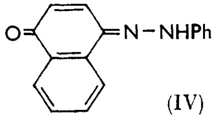
[26] Brode, ref. 9, p. 8.

Initially there will be much more B than C in the solution, and the points of spectral intersection will be determined by A and B only. However, as reaction proceeds y increases and the optical densities at the initial crossing wavelength, λ^1, will be less than or greater than $D_A{}^1$ according as $\varepsilon_C{}^1$ is less or greater than $\varepsilon_A{}^1$, *i.e.*, as $(\varepsilon_C{}^1 - \varepsilon_A{}^1)$ in equation (10) is negative or positive. Further, the shift in absorption will be monotonic with increasing values of y, and therefore with time. It should be noted that consecutive reactions do not have a uniquely defined ξ.

A recent example is given by Fischer and Frei,[27] in their study of tautomerism and geometrical isomerism of 4-phenylazo-1-naphthol (III). It was shown there that the *cis*-isomer is not spontaneously converted directly into the *trans*-isomer, but rather into the tautomer (IV), which in turn is transformed into the *trans*-azo-compound. We thus have the sequence *cis*-(III) ⟶ (IV) ⟶ *trans*-(III), in analogy with $A \longrightarrow B \longrightarrow C$ above. Fig. 4b describes this reaction. The *trans*-phenol (III) and the hydrazone (IV) are characterized by maxima at 416 and 482 mμ, respectively. During most of the reaction the behaviour is as predicted by equation (10); in the last stages of the reaction, however, there is no more *cis*-isomer present, and an isosbestic point is observed, characterizing the binary system (IV) + *trans*-(III).



(III)



(IV)

Brode [26] gives some relevant examples. In bisazo-dyes, if the azo-groups are essentially isolated from one another, the absorption is determined by the total number of *cis*- and the total number of *trans*-groups. If there is no other absorbing species, this case is essentially an example of $A \rightleftharpoons B$ behaviour and isosbestic points are observed. If, on the other hand, the azo-groups are in conjugation with one another there will be *trans-trans*-, *cis-trans*-, and the *cis-cis*-species, each with a different spectrum. Further, if the course of reaction is *trans-trans* ⟶ *cis-trans* ⟶ *cis-cis*, as seems probable, the system should not show isosbestic points. This is indeed the case (Brode does not give specific examples but describes the type of behaviour to be expected in the general case). It should be noted that the absence of isosbestic points cannot be attributed only to the system's being a three-component one; it follows, rather, from the fact that the reactions are consecutive.

Wald and Brown [28] studied the synthesis and photochemical bleaching of the eye-pigment rhodopsin. During the synthesis, accomplished by incubating neo-b-retinene with the rod-protein opsin, the spectra of the system have an isosbestic point. This the authors take to indicate that only two of the molecular species present absorb at the wavelength of this point; our arguments indicate that this conclusion is not necessarily valid. On photochemical bleaching, rhodopsin is transformed into opsin and all-*trans*-retinene, without an isosbestic point. This is attributed to the complexity of the process that involves intermediate retinenes. In a later paper [29] it was shown that this process involves, in fact, a primary photochemical step, followed, at room temperature, by two consecutive thermal reactions.

Fluorescence Spectra.—Because of the marked difference in quenching characteristics often found between even structurally similar molecules, one does not, in general, expect to find isosbestic points in fluorescence spectra. Nevertheless, examples of such points

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[27] Fischer and Frei, *J.*, 1959, 3159.

[28] Wald and Brown, *Nature*, 1955, **177**, 174.

[29] Hubbard, Brown, and Kropf, *Nature*, 1959, **183**, 442.

are known. Thus, an isosbestic point is found in the fluorescence spectra of aqueous solutions of β-naphthol as the pH is varied.[30]

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[30] Weller, in "Progress in Reaction Kinetics," ed. Porter, Pergamon Press, Oxford, Vol. I, 1961, p. 201.